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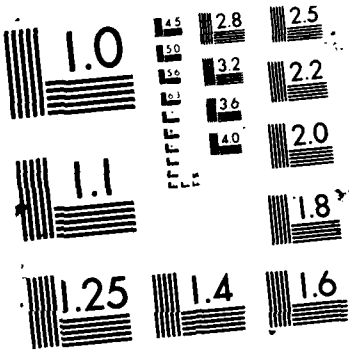
1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6
6.3

SYNTHESIS OF TWO COMPONENT MODELS OF ELASTIN

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Morphologically elastic fibers can be described as a fine fibrillar coating of a large amorphous core referred to as elastin. Elastin is an insoluble, highly cross-linked and very hydrophobic protein with about 90% non-polar amino acids and about 5% lysines. The insolubility of elastin is due to the presence of cross-links, primarily desmosine and isodesmosine (Figure 1), which are formed from four lysine residues, two each from two different peptide chains. The cross-linking sequences KAAAK and KAAK were observed to repeat at least six times in the soluble precursor protein, tropoelastin, which is comprised of 800-850 amino acids. Determination of the amino acid sequence of porcine tropoelastin using tryptic peptides is 80% complete[1]. The largest porcine tryptic peptide is 81 residues; the dominant feature of this peptide is the repeating pentapeptide sequence $(Val\text{-}Pro\text{-}Gly\text{-}Val\text{-}Gly)_n$ with n = 11+ in pig. Conformational studies on oligo-and polypentapeptides of the above repeating sequence, carried out in this laboratory, resulted in the development of a new class of conformations called β-spirals in which a β-turn occurs with regularity along the helical axis. A new mechanism of elasticity, "a librational entropy mechanism," has been put forward to explain the elastic behavior of the polypentapeptide[2,3,4]. This contrasts with the "random chain-network theory" previously proposed for elastin[5]. Demonstration of the librational entropy mechanism and the non-random nature of elastin has been achieved by numerous physical characterizations: light and electron microscopy, circular dichroism, Raman spectroscopy, dielectric relaxation, nuclear magnetic resonance, temperature dependence of length, of force development and of elastic modulus and composition studies[6].

In order to develop polypeptides as models for the natural insoluble elastin, it is useful to synthesize a polypentapeptide molecule with cross-linking sequences and then polymerize them to yield very high molecular weight polymers which on enzymatic cross-linking by lysyl-oxidase could result in biomaterials with physical properties similar to elastin.

Here we report the synthesis of the two polymers $[XL\text{-}1\text{-}(VPGVG)_{15}]_n$ and $[XL\text{-}2\text{-}(VPGVG)_{15}]_n$ where XL-1 is the cross-linking sequence, AAAAKAAKYGA and XL-2 is the second cross-linking sequence, AAKAAAKAA.

Synthesis: The synthesis of the two monomeric peptides, AAAAKAAKYGA-$(VPGVG)_{15}$ and AAKAAAKAA-$(VPGVG)_{15}$ was carried out by the solid phase methodology[7] (Scheme I). The first pentamer sequence VPGVG was built on the 1% cross-linked Merrifield resin by adding a single amino acid at a time. The next 14 pentamer units were attached by the segment condensation approach using Boc-VPGVG-OH, synthesized by the classical solution methods as reported earlier[8]. The amino acids in the cross-linking sequences were once again coupled by stepwise condensation. The side chain functional groups of Lys and Tyr were protected by Cbz and O-Br-Cbz groups respectively. The segment condensations were carried out in DMF, TFE-CH_2Cl_2 or TFE alone in the presence of HOBt. Occasionally preformed symmetrical anhydrides of individual amino acids had to be used to ensure the completeness of the reaction. In order to check the progress of the coupling reactions, the peptides were removed from the resin as methyl esters at various stages of peptide synthesis when there were 6, 9, 12 and 15 pentamer units attached to the growing peptide chain. An approximate determination of the peptide chain length during the course of the synthesis could be obtained from a plot of ln(molecular weight) versus the midpoint of the temperature profile of turbidity (T_{P_T}) as well as from NMR end group analysis[9]. After the synthesis was completed on the resin, the Boc-protected peptides were removed as methyl esters by transesteri-fication and purified by repeated precipitations from different solvent systems. The purity of the peptide was checked by C-13 magnetic resonance spectra and amino acid analysis. After saponification, the peptides were converted to p-nitrophenyl esters by reacting with excess of bis (p-nitrophenyl) carbonate[10] for several days.

The Boc-group was removed and the peptides were polymerized for 8 - 12 weeks in dimethylsulfoxide in the presence of N-methylmorpholine. After diluting with water, the polymers were dialyzed against water using a 50kD cut-off dialysis tubing for 15 days and the retentates were lyophilized. The purity of the polymers was again checked by CMR (Figure 2) and amino acid analysis.

In conclusion, the feasibility of synthesizing large peptides of 86 and 84 amino acid long and polymerizing them into polymers having molecular weights of greater than 50kD is demonstrated. The next step will be to remove the Z groups on lysines, submit the polymers separately and mixed to lysyl oxidase treatment, study the various intermediate oxidation products and also the formation of final desmosine and isodesmosine structures, and compare the mechanical properties of the insoluble matrix to those of natural elastin.

ACKNOWLEDGEMENT:

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References:

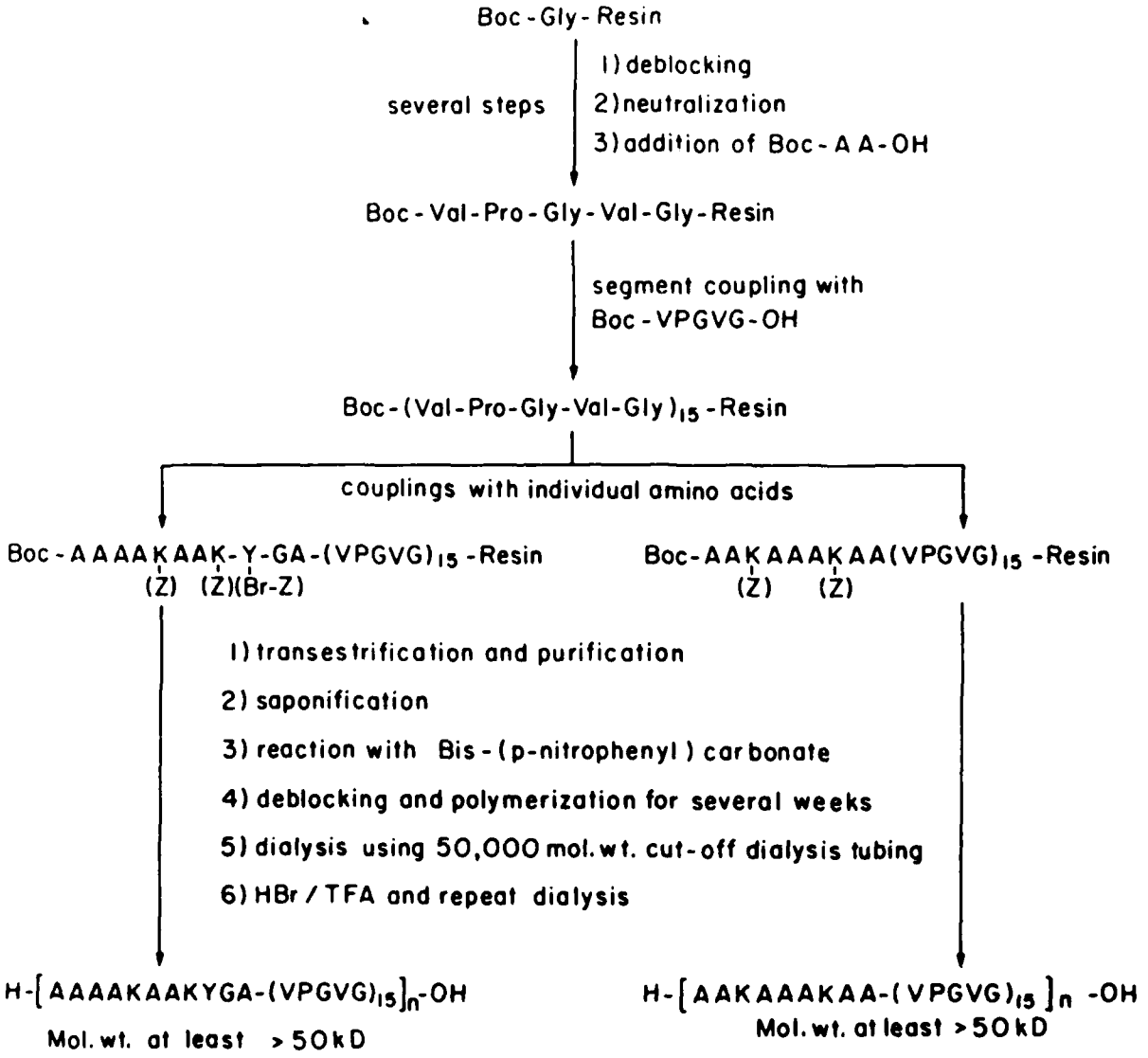
1. Sandberg, L.B., Leslie, J.G., Leach, C.T., Alvarez, V.L., Torres, A.R. and Smith, D.W., Path. Biol., 33(1985) 266.
2. Urry, D.W., In Cunningham, L.W. and Frederiksen, D.W. (Eds.) Methods in Enzymology, Academic Press, Inc., New York, 1982, p. 673.
3. Urry, D.W., Venkatachalam, C.M., Long, M.M. and Prasad, K.U., In Srinivasan, R. and Sarma, R.H. (Eds.) Conformation in Biology, Adenine Press, USA, 1982, p. 11.
4. Urry, D.W. and Venkatachalam, C.M., Int. J. Quantum Chem.: Quantum Biol. Symp. No. 10(1983) 81.
5. Hoeve, C.A.J. and Flory, P.J., Biopolymers, 13(1974) 677.
6. Urry, D.W., In Robert, L. and Hornebeck, W. (Eds.) Elastin and Elastases, CRC Press, Boca Raton (in press).
7. Merrifield, R.B., J. Am. Chem. Soc., 85(1963) 2149.
8. Prasad, K.U., Iqbal, M.A. and Urry, D.W., Int. J. Pept. and Protein Res., 25(1985) 408.
9. Urry, D.W., Trapane, T.L. and Prasad, K.U., Biopolymers, 24(1985) 2345.
10. Wieland, Th., Heinke, B. and Vogeler, J., Ann., 655(1961) 189.

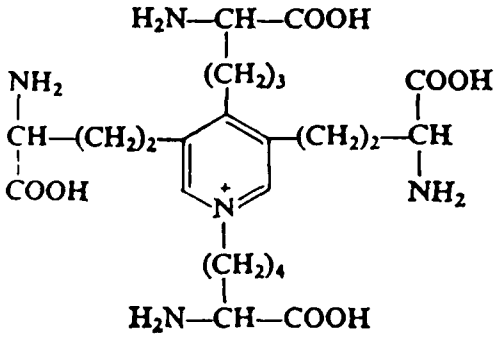
Figure Legends:

Figure 1 Structures of desmosine and isodesmosine.

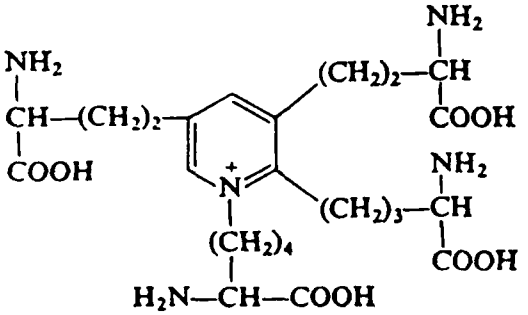
Figure 2 CMR Spectra of (A) Poly [XL-1-$(VPGVG)_{15}$], (B) Poly [XL-2-$(VPGVG)_{15}$] and (C) Poly (VPGVG) at 25 MHz in dimethylsulfoxide.

Synthesis of Elastin Peptide Models with Cross-Linking Sequences





Desmosine



Isodesmosine

FIGURE 1

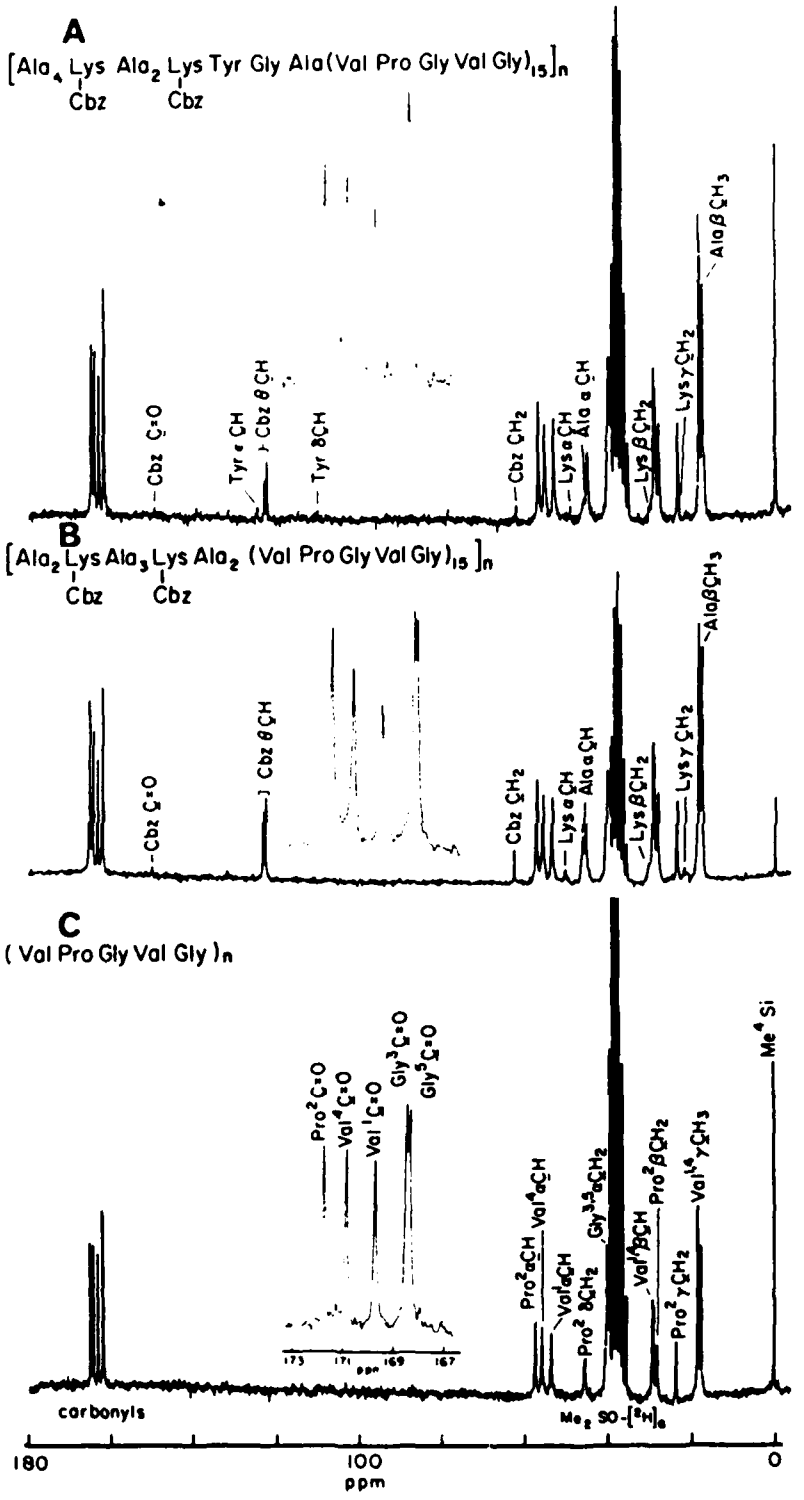

A
[Ala4 Lys Ala2 Lys Tyr Gly Ala(Val Pro Gly Val Gly)15]n
Cbz
Cbz
Cbz C=O
Tyr αCH
Cbz βCH
Tyr βCH
Cbz CH2
Lys αCH
Ala αCH
Lys βCH2
Lys γCH2
Ala βCH3
B
[Ala2 Lys Ala3 Lys Ala2 (Val Pro Gly Val Gly)15]n
Cbz
Cbz
Cbz C=O
Cbz βCH
Cbz CH2
Lys αCH
Ala αCH
Lys βCH2
Lys γCH2
Ala βCH3
C
(Val Pro Gly Val Gly)n
Pro2 C=O
Val4 C=O
Val1 C=O
Gly3 C=O
Gly5 C=O
Pro2 αCH
Val4 αCH
Val1 αCH
Pro2 βCH2
Gly3,5 αCH2
Val4 βCH
Pro2 βCH2
Pro2 γCH2
Val1,4 γCH3
Me4 Si
173
171
169
167
ppm
carbonyls
Me2 SO-[2H]6
180
100
0
ppm

FIGURE 2

END

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DTIC